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Correlation Between CD34 Expression and Chromosomal Abnormalities but not Clinical Outcome in Acute Myeloid Leukemia

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The hemopoietic stem cell marker CD34 has been reported to be a useful predictor of treatment outcome in acute myeloid leukemia (AML). Previous data suggested that CD34 expression may be associated with other poor prognosis factors in AML such as undifferentiated leukemia, secondary AML (SAML), and clonal abnormalities involving chromosome 5 and 7. In order to analyze the correlations between the clinicopathologic features, cytogenetic and CD34 expression in AML, we retrospectively investigated 99 patients with newly diagnosed AML: 85 with de novo disease and 14 with secondary AML (SAML). Eighty-six patients who received the same induction chemotherapy were available for clinical outcome.

Defining a case as positive when ≥20% of bone marrow cells collected at diagnosis expressed the CD34 antigen, forty-five patients were included in the CD34 positive group. Ninety patients had adequate cytogenetic analysis. Thirty-two patients (72%) with CD34 positive AML exhibited an abnormal karyotype whereas 15 patients (28%) with CD34 negative AML had abnormal metaphases ($P < 0.01$). Monosomy 7/7q- or monosomy 5/5q- occurred in 10 patients and 8 of them expressed the CD34 antigen ($P < 0.05$). All patients with t(8;21) which is considered as a favorable factor in AML had levels of CD34 ≥ 20% ($P < 0.05$). We did not find any association between CD34 expression and attainment of complete remission, overall survival, or disease-free survival.

In conclusion, the variations of CD34 expression in AML are correlated with cytogenetic abnormalities associated both with poor and favorable outcome. The evaluation of the correlations between CD34 antigen and clinical outcome in AML should take into account the results of pretreatment karyotype.

Key words: CD34 antigen, chromosomal abnormalities, acute myeloid leukemia

INTRODUCTION

Prognostic criteria are useful to aid in the choice of the most appropriate therapy for patients with hematologic malignancies. Over the past decade several prognostic factors based upon FAB classification, immunophenotyping, cytogenetics, and clinical characteristics have been described in acute myeloid leukemia (AML). Previous investigations suggested that the expression of CD34 antigen on blast cells defined a poor prognosis subset of patients with AML [1–4].

The CD34 antigen is a membrane glycoprotein present on 1–2% of normal bone marrow cells which is expressed on hemopoietic stem and progenitor cells [5]. The frequency of CD34 expression varied from 45 to 65% in cases of AML [1–4]. Comparison between CD34 positive and CD34 negative leukemia show that CD34 expression was correlated with known poor prognostic factors such as less differentiated leukemia, previous chemoradiotherapy exposure, or chromosomal abnormalities involving

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TABLE I. Comparative Characteristics of Patients With CD34 Positive and Negative AML*

	CD34+	CD34−	*P*
Number	45	54	
Age mean (range)	55.8 (23–78)	51.9 (19–89)	NS
Age >55 years	24	23	NS
Male/female	25/20	24/30	NS
SAML	13	1	<0.01
WBC mean (range)	29.7 (0.3–214)	50.3 (1–264)	<0.05
FAB			
M1	12	15	NS
M2	15	7	<0.02
M3	2	9	NS
M4	7	16	NS
M5	1	2	NS
M6	2	3	NS
M7	0	0	NS
M0	6	2	NS
Karyotype			
No metaphase obtained	5	4	NS
Abnormal	32	15	<0.01
t(15;17)	2	9	NS
+8	4	3	NS
-7/7q- or -5/5q-	8	2	<0.05
t(8;21)	5	0	<0.05

*NS, not significant.

TABLE II. Clinical Outcome of the 86 Evaluable Patients as a Function of CD34 Expression*

	CD34+	CD34−	*P*
Number	35	51	NS
Induction phase			
Early death	4	9	NS
Primary refractive	7	11	NS
Complete remission	24	31	NS
Relapse	16	24	NS
Median survival (months)	15	13	NS
Median duration of CR (months)	14	18	NS

*NS, not significant.

chromosomes 5 and 7 [1–4]. If the correlation between this immaturity marker and minimally differentiated leukemia could be expected, in some studies the CD34 antigen was highly expressed on more differentiated AML (M2) [3,4]. Furthermore, more recent studies have not confirmed the prognostic value of the CD34 expression [6–8].

Therefore, the interpretation of the biologic and clinical relevance of CD34 expression remains much debated. To further analyze the clinicopathologic features and cytogenetic profile of CD34 positive AML, we investigated 99 patients with newly diagnosed AML.

MATERIALS AND METHODS

Patient Population and Treatment

Ninety-nine adult patients with previously untreated AML seen at the Centre Henri Becquerel, Rouen, France, between January 1985 and January 1994 were included in the present report. Patients were selected on the basis of cell availability. Patients with previous exposure to chemotherapy and/or radiation, or a history of hematologic disorder (SAML), were not excluded from the study. In 86 cases, the induction treatment consisted of conventional chemotherapy with an anthracycline (D1-D3) and intermediate-dose of cytarabine (D1-D7). Ten patients older than 70 years did not receive intensive chemotherapy and 3 patients with AML M3 were treated with all-trans retinoic acid (ATRA). For the 58 patients who obtained a complete remission (CR), it was followed by an allogeneic bone marrow transplantation (BMT) in 5 cases, of autologous BMT in 3 patients, and a consolidation chemotherapy in 50 cases.

Immunophenotyping

A bone marrow sample was collected at diagnosis for cytologic, immunologic, and cytogenetic analysis. AML were classified according to the FAB classification [9].

Before 1992 an aliquot of leukemic cells was frozen to allow the immunophenotype study. After this date, the immunophenotype was performed on fresh cells. Mononuclear cells were separated from heparinized marrow by Ficoll-Metrizoate gradient. Surface membrane antigens were detected by the standard direct immunofluorescence method using fluorescein isothiocyanate (FITC) or phycoerythrin (PE) conjugated monoclonal antibodies. Reactivity was tested with a panel of monoclonal antibody purchased from Immunotech (Luminy, France): CD13 (SJ1D1), CD14 (R1052), CD15 (80H5), CD33 (D3HL60251), CD34 (QBEND10), CD38 (T16). Negative controls substituting the MoAbs by irrelevant immunoglobulin of the same isotype were included. Cells were counted by flow cytometry (EPICS CS cell sorter, Coulter system, Coulter Corp., Miami, FL) analyzing 5×10^3 cells at 488 nm. A positive reaction was defined as 20% of gated cells being fluorescent.

Cytogenetic Analysis

Bone marrow samples were suspended at a concentration of 2×10^6 cells/ml in RPMI 1640 medium supplemented with 10% fetal calf serum. Cells were cultured overnight in the presence of colchicine (0.02 g/ml). Preparations were incubated for 25 min at 37°C in 0.075 mol/l potassium chloride, fixed in methanol-acetic acid (3:1), and spread on clean dry slides. R-banding was performed according to Sehested method [10]. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature [11].

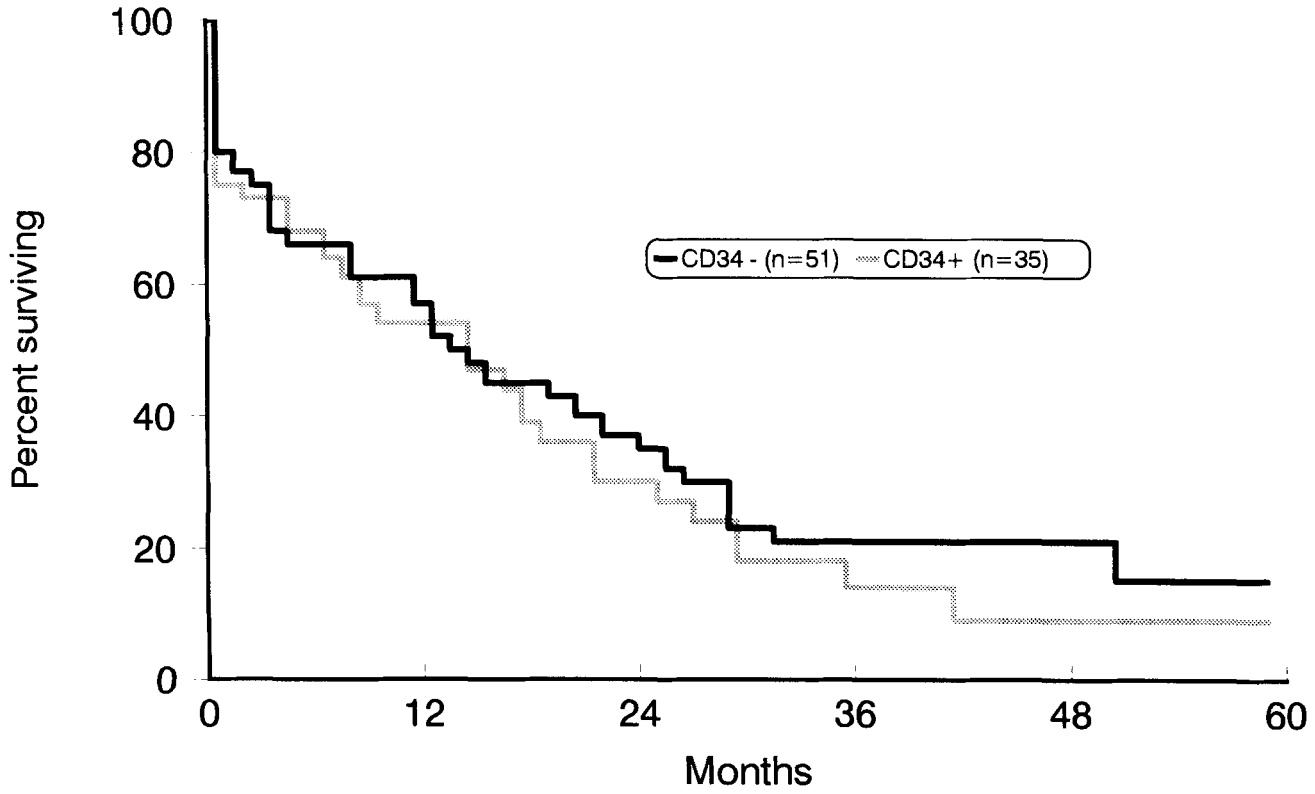

Fig. 1. Kaplan and Meier plots for overall survival according to the CD34 expression on blast cells from the 86 patients with AML who received a conventional induction chemotherapy. The median survival was 15 months for the CD34+ AML patients and 13 months for the CD34− patients. There was no significant association between CD34 expression and survival.

Statistical Analysis

Overall survival was measured from the date of diagnosis until death or last follow-up. Duration of CR was evaluated from the date of CR achievement until relapse, death, or last follow-up. Associations between qualitative covariates (sex, disease categories, FAB subtype, immunophenotype, chromosome analysis) were tested using Chi-square test with Yates correction [12]. Association between quantitative (Age, WBC counts) and qualitative covariates were analyzed by Student's *t*-test. Survival and disease-free survival were based on the Kaplan and Meier estimate [13] and compared with the Log Rank test [14]. The patients who did not receive an intensive chemotherapy as well as patients treated by ATRA were excluded from the survival analysis. Analyses were performed using the JPSI statistical software (developed by P. Kwiatkowski, Centre Jean Perrin, Clermont-Ferrant, France).

RESULTS

Immunophenotyping and cytogenetic analysis were performed on 99 patients. In all cases the leukemic cells expressed at least one of the antigens associated with myeloid differentiation (CD13, CD14, CD15, CD33). The more commonly detected markers were CD33 (73%) and CD13 (68%). Forty-five patients had more than 20% blasts expressing CD34 antigen. Comparative characteristics of the patients in CD34 positive and CD34 negative groups are listed in Table I. There was no difference in age and sex between the two groups. Patients with CD34 positive AML had a significantly higher incidence of secondary AML ($P < 0.01$) and lower WBC count ($P < 0.05$) than CD34 negative AML. CD34 was detected in a majority of FAB M2 (68%) cases ($P < 0.02$) and in FAB MO (72%) cases. CD33 was significantly less expressed in CD34 positive AML (mean = 38.8± 27.5%) than CD34 negative AML (mean = 62.3 ± 27.27%) ($P < 0.01$).

Cytogenetic Studies

Chromosomes could be successfully analyzed in 90 patients. Forty-seven of them had abnormal metaphases. The most frequent modal number was 46 in 28 cases. Fourteen were hyperdiploid and 5 hypodiploid. A mixture of normal and clonally abnormal cells was present in 10

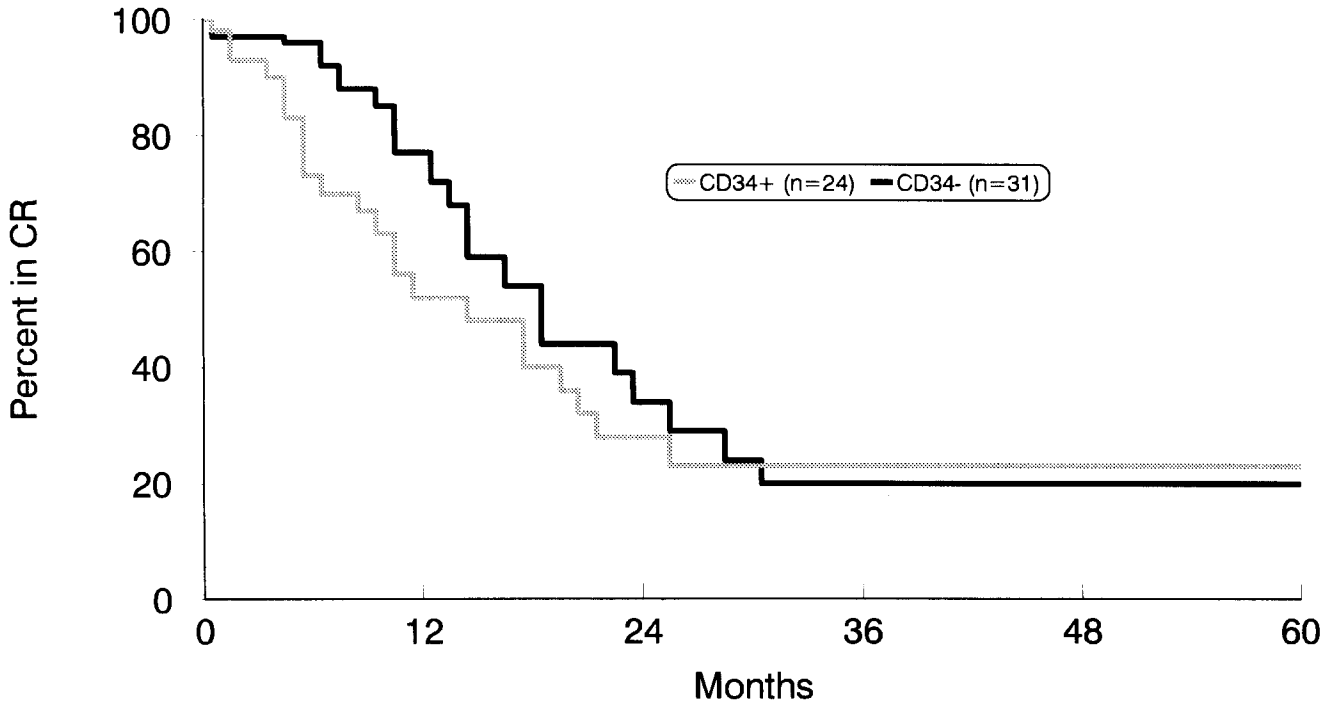

Fig. 2. Kaplan and Meier plots for CR duration according to CD34 expression in the 55 patients who achieved a CR. The median CR duration was 18 months for the CD34+ AML patients and 14 months for the CD34− AML patients. There was no significant association between the expression of CD34 and the disease-free survival.

cases whereas karyotype exhibited only abnormal metaphases in 37 samples. The most common cytogenetic aberrations were in decreasing order of frequency: t(15;17)(q22;q25) (n = 11), trisomy 8(+8) (n = 7), monosomy 7 or 7q- (-7/7q-) (n = 7), monosomy 5 or 5q- (-5/5q-) (n = 5), t(8;21)(q22;q22) (n = 5).

Cytogenetic findings based on CD34 expression are summarized in Table I. Karyotype was abnormal in 32 of the 45 patients with CD34 positive AML and in 15 of the 54 patients with CD34 negative AML ($P < 0.01$). The t(15;17) and +8 occurred in CD34 positive and CD34 negative AML. The majority of patients with t(15;17) (82%) did not express the CD34 antigen. Monosomy 7/7q- or -5/5q- were identified in 10 patients including 7 patients with a complex karyotype. Among them, 8 were included in the CD34 positive AML group ($P < 0.05$). The t(8;21) occurred in five patients. They all belonged to the CD34 positive group. The t(8;21) was associated to a lost chromosome Y in 2 patients and a duplication of the short arm of the chromosome 9 (dup9p) in one patient. Within the M2 AML, CD33 antigen was significantly less expressed in patients bearing t(8;21) (21% ± 10) than other M2 AML (55% ± 25) ($P < 0.025$).

Clinical Outcome

Response to therapy and survival was available in 86 patients. Fifty-five patients (62%) entered CR. The median survival for all patients was 14 months. In univariate analysis, only age >55 years was significantly associated with a poor survival ($P < 0.00001$) and a shorter CR duration ($P < 0.01$).

There was no association (Table II) between CD34 expression and induction failures, attainment of CR, relapse, overall survival (Fig. 1) or disease-free survival (Fig. 2). The 10 patients bearing at least -7/7q- or -5/5q- had a very poor outcome. Eight received conventional induction chemotherapy, the median survival in this small subgroup was 4 months and 5 patients died within 7 months. The 5 patients with M2 AML and t(8;21) were treated with conventional induction chemotherapy. A CR was obtained in all patients. Two patients died from relapse after 1 year of remission, and three patients are still alive in first remission 15, 24, and 59 months after the diagnosis.

DISCUSSION

The hemopoietic stem cell marker CD34 is expressed on about 50% of AML cases, mainly of the undifferentiated FAB subtype or even unclassifiable morphologic subtype [1,3,15]. It has been demonstrated that patients suffering from minimally differentiated AML were unresponsive to chemotherapy and had shorter survival [15,16]. Previous investigations suggested therefore that CD34 expression might define a poor prognostic subsets

of patients [1–4,17]. We retrospectively investigated 99 patients with newly diagnosed AML, 85 with de novo AML, and 14 with SAML. Forty-five patients expressed the CD34 antigen on their blast cells.

Our study showed that CD34 positivity was, respectively, associated with the disease category (SAML), abnormalities of chromosomes 5 or 7 and FAB subtype M2 especially those bearing the t(8;21). The frequent occurrence of aberrations involving chromosome 7 in CD34 positive AML has been recognized since 1988 by Vaughan et al. [18]. Borowitz et al. [1] and Geller et al. [2] demonstrated later that SAML or AML with chromosome 5 or 7 abnormalities had a higher expression of the CD34 marker. Chromosome 5 or 7 abnormalities are frequently involved in SAML [19,20]. If a stem cell marker positivity in SAML could be expected considering that the target cell is a multipotent cell [21], it is more surprising to observe in most studies a high level of CD34 expression on more cytologically differentiated leukemic cells (M2) [1,3,4]. As previously described by Kita et al. [22], we found that M2 AML bearing the t(8;21) expressed the CD34 antigen and had a lower CD33 expression indicating that this translocation might distinguish a subgroup of M2 AML with early hemopoietic development.

The most recent studies including our own did not confirm the correlation between a high level of CD34 expression and a poor prognosis in AML. It has been demonstrated that AML with chromosome 5 and 7 abnormalities are usually refractory to chemotherapy and have a short survival [23] whereas AML with t(8;21) have good response to chemotherapy with a high remission rate and long survival [24–26]. The presence in CD34 positive AML of cytogenetic abnormalities usually associated with both poor and favorable outcome could explain the lack of correlation between CD34 expression and survival in our experience. In most studies CD34 expression was associated with poor prognostic factors of AML such as disease category (de novo vs. SAML), MO subtype, or chromosome 5 and 7 abnormalities [1,2,4,15,16]. This correlation may explain the prognostic value of CD34 reported previously. Two studies [2,17] suggested that CD34 expression was one of the most important predictors for response to therapy in multivariate analysis. But when Geller et al. [2] took into account the disease categories, the statistical significance disappeared. Solary and coworkers [17] did not include cytogenetic data in his statistical analysis.

In conclusion, these data, based on a series of 99 patients with AML, confirmed the association of the CD34 antigen with SAML and FAB subtype M2. We demonstrated that expression of CD34 at diagnosis does not influence the prognosis when patients are treated uniformly with cytarabine and anthracycline. The CD34 expression is correlated with cytogenetic abnormalities, which are known as one of the most important prognostic factors in AML [23,27–29].

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